FIG. 1

PMP-2 10 20 30 40 50 60 70

H_2N-SDDPKESEGDLHCVCVKTTSLVRPRHITNLELIKAGGHCPTANLIATKKNGRKLCLDLQAALYKKKIIKKLLES-COOH

Cationic

Native Sequence: ALYKKKIIKKLLES

Modeling:

Cationic Cationic

RP-1 Sequence: ALYKKFKKKLLKSLKRLG

09648816.082500

FIG. 2A

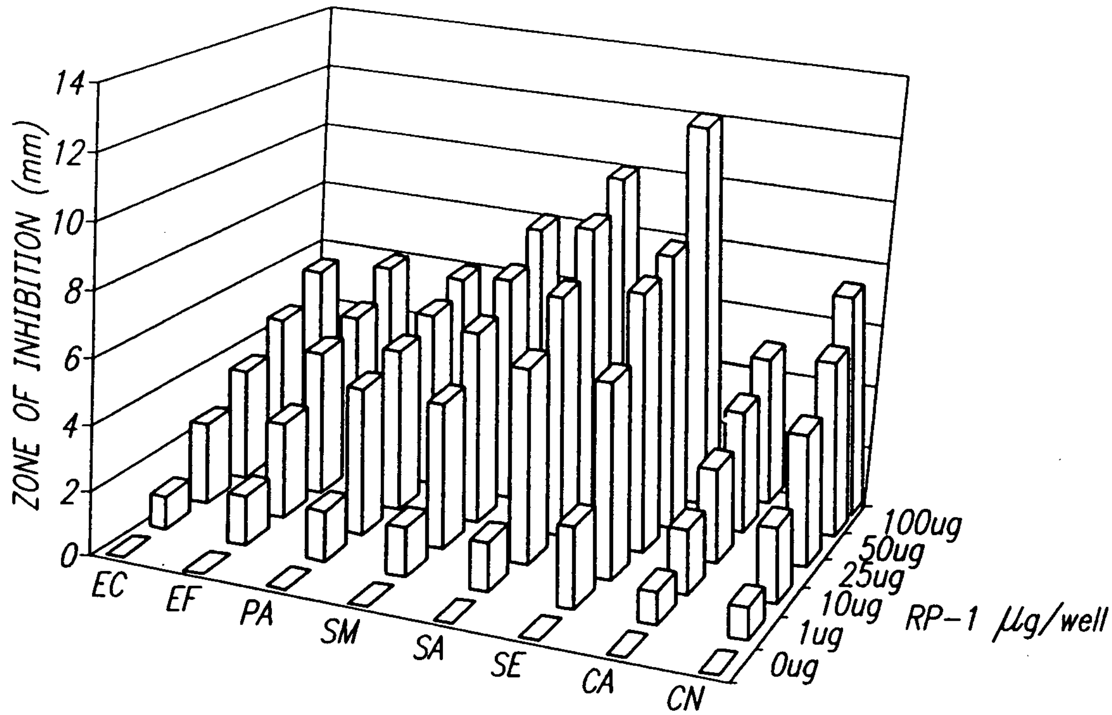

ZONE OF INHIBITION (mm)
0
2
4
6
8
10
12
14
EC
EF
PA
SM
SA
SE
CA
CN
100ug
50ug
25ug
10ug
1ug
0ug
RP-1 μg/well

FIG. 2B

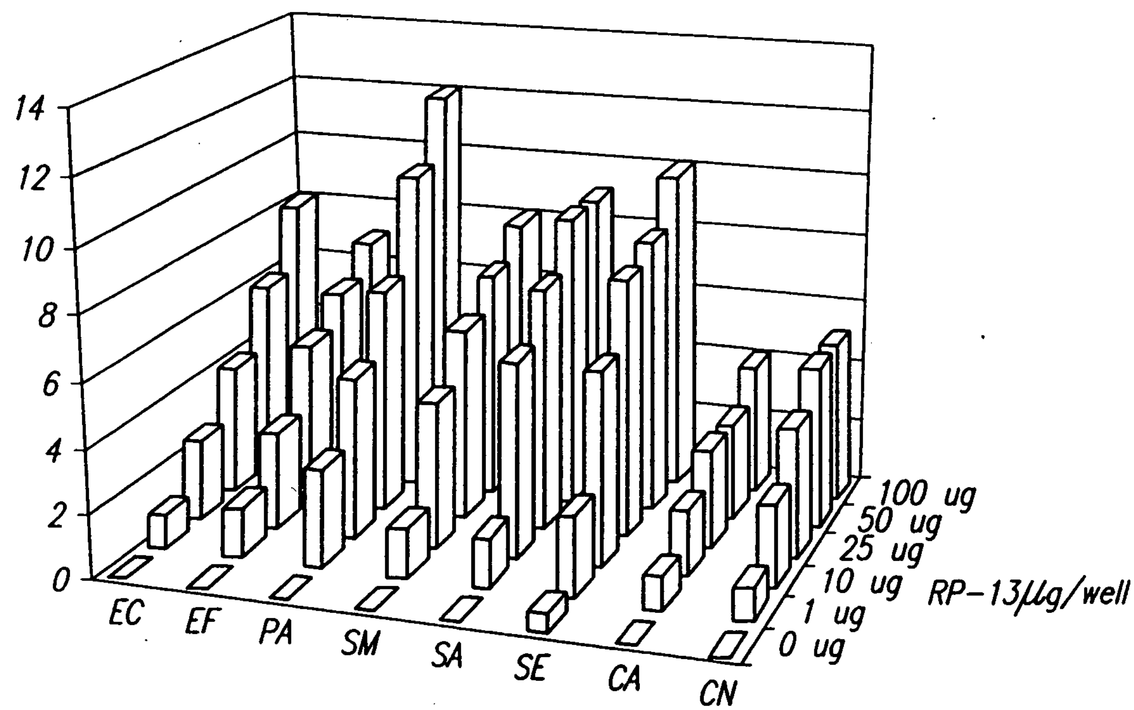
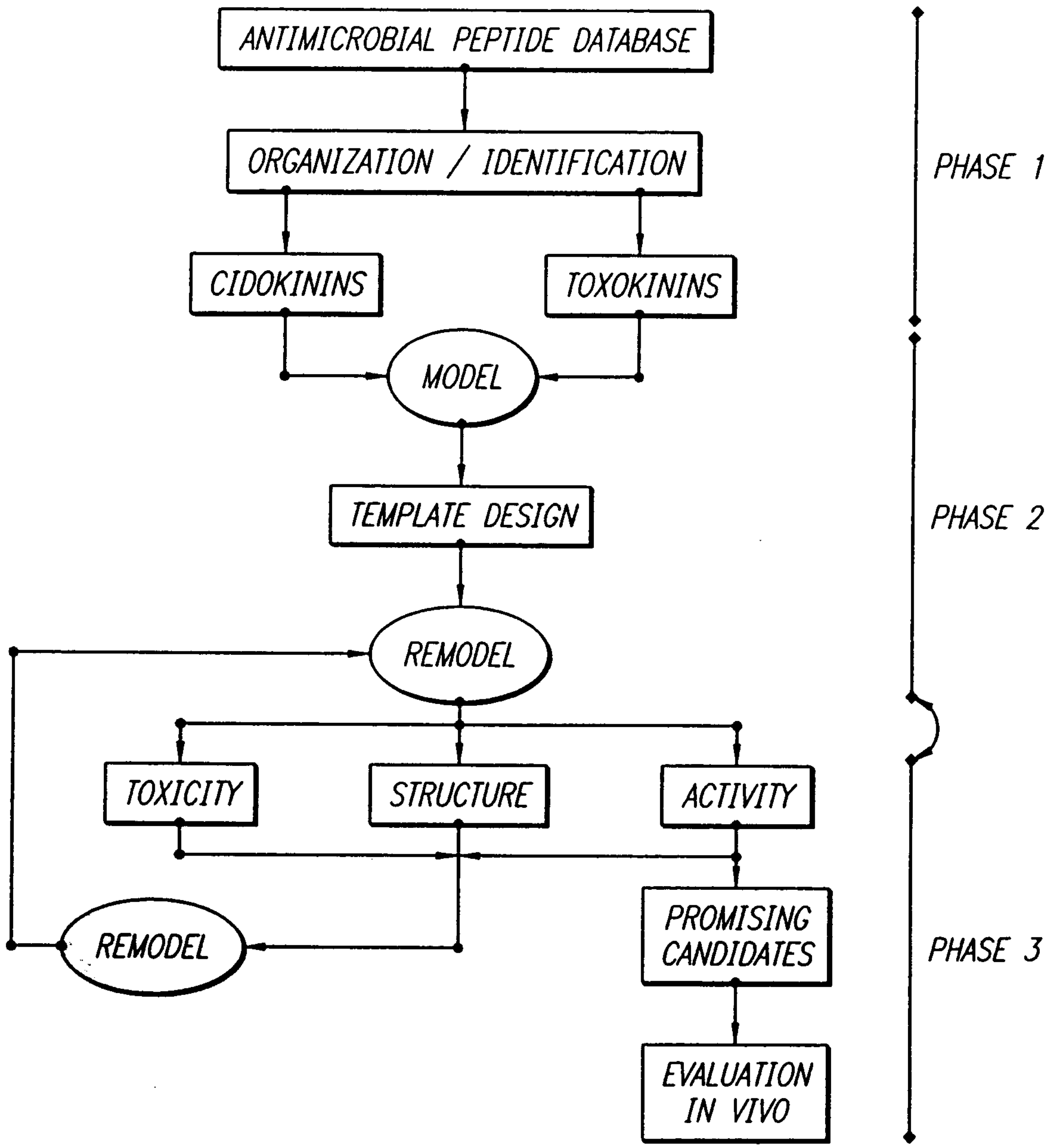

0
2
4
6
8
10
12
14
EC
EF
PA
SM
SA
SE
CA
CN
100 ug
50 ug
25 ug
10 ug
1 ug
0 ug
RP-13μg/well

FIG. 3



ANTIMICROBIAL PEPTIDE DATABASE
ORGANIZATION / IDENTIFICATION
CIDOKININS
TOXOKININS
MODEL
TEMPLATE DESIGN
REMODEL
TOXICITY
STRUCTURE
ACTIVITY
PROMISING CANDIDATES
REMODEL
EVALUATION IN VIVO
PHASE 1
PHASE 2
PHASE 3

00520280 . 91884960

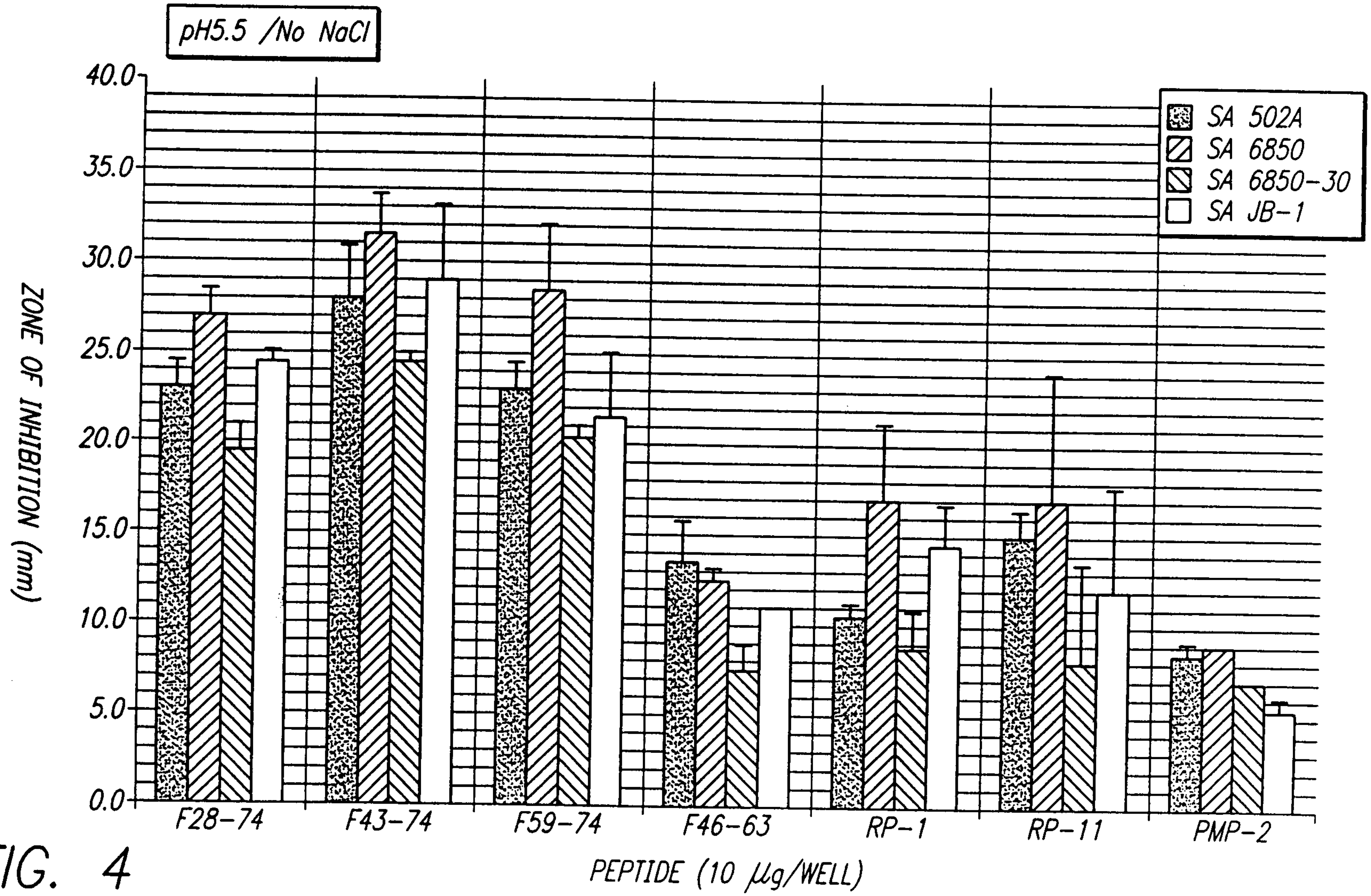
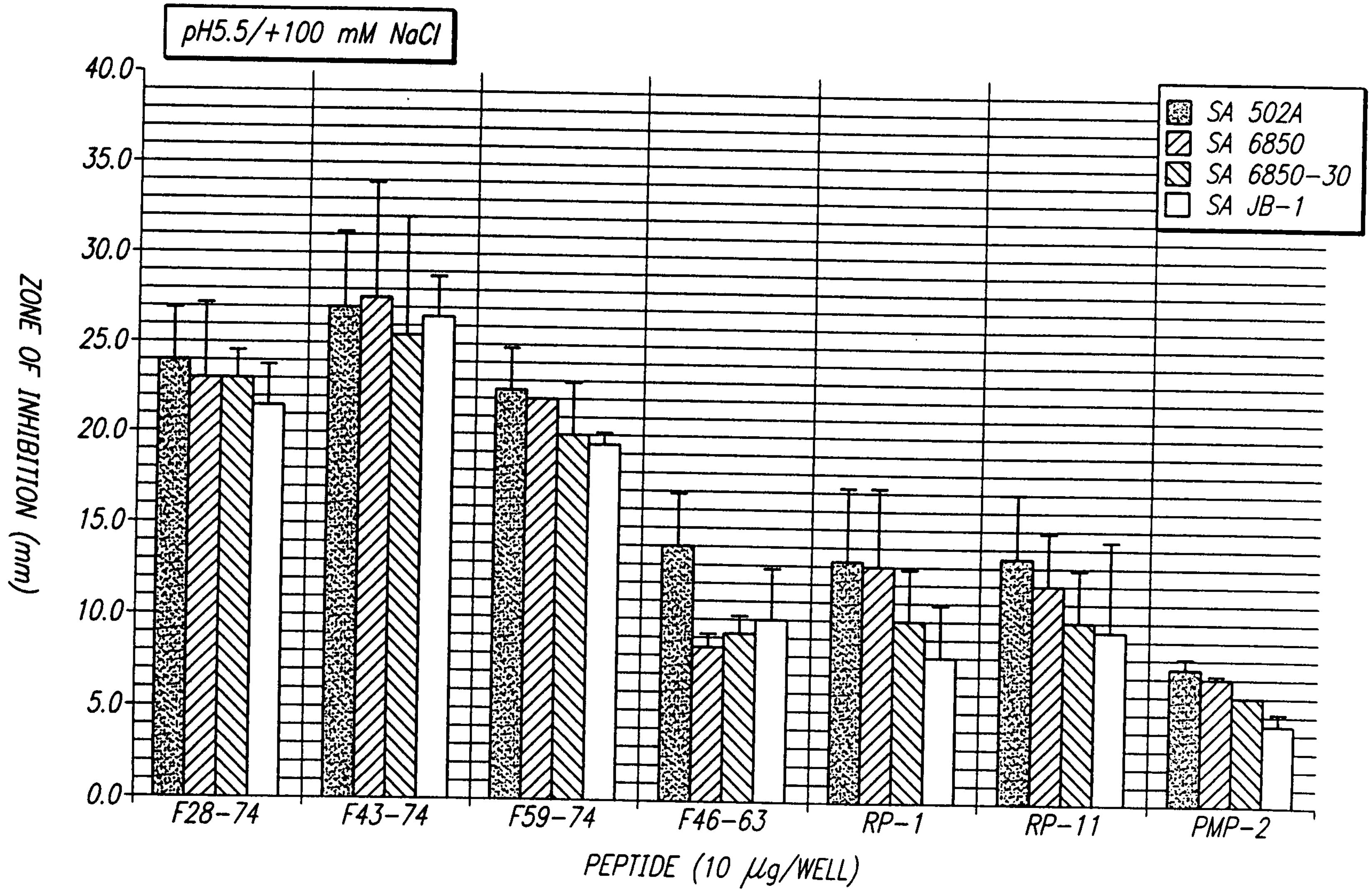


FIG. 4

FIG. 5


pH5.5/+100 mM NaCl
SA 502A
SA 6850
SA 6850-30
SA JB-1
ZONE OF INHIBITION (mm)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
F28-74
F43-74
F59-74
F46-63
RP-1
RP-11
PMP-2
PEPTIDE (10 μg/WELL)

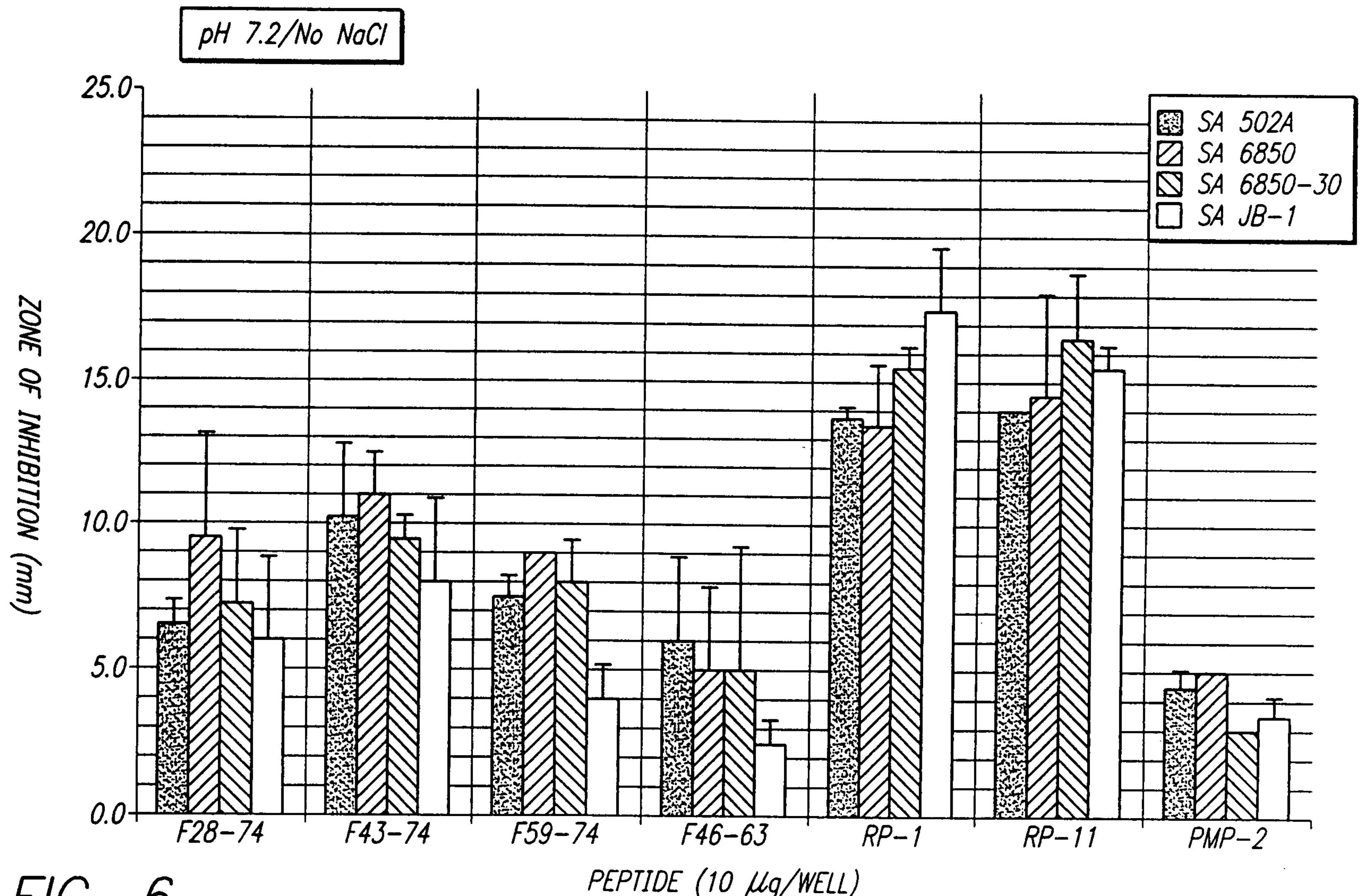

pH 7.2/No NaCl
ZONE OF INHIBITION (mm)
25.0
20.0
15.0
10.0
5.0
0.0
SA 502A
SA 6850
SA 6850-30
SA JB-1
F28-74
F43-74
F59-74
F46-63
RP-1
RP-11
PMP-2
PEPTIDE (10 μg/WELL)

FIG. 6

FIG. 7

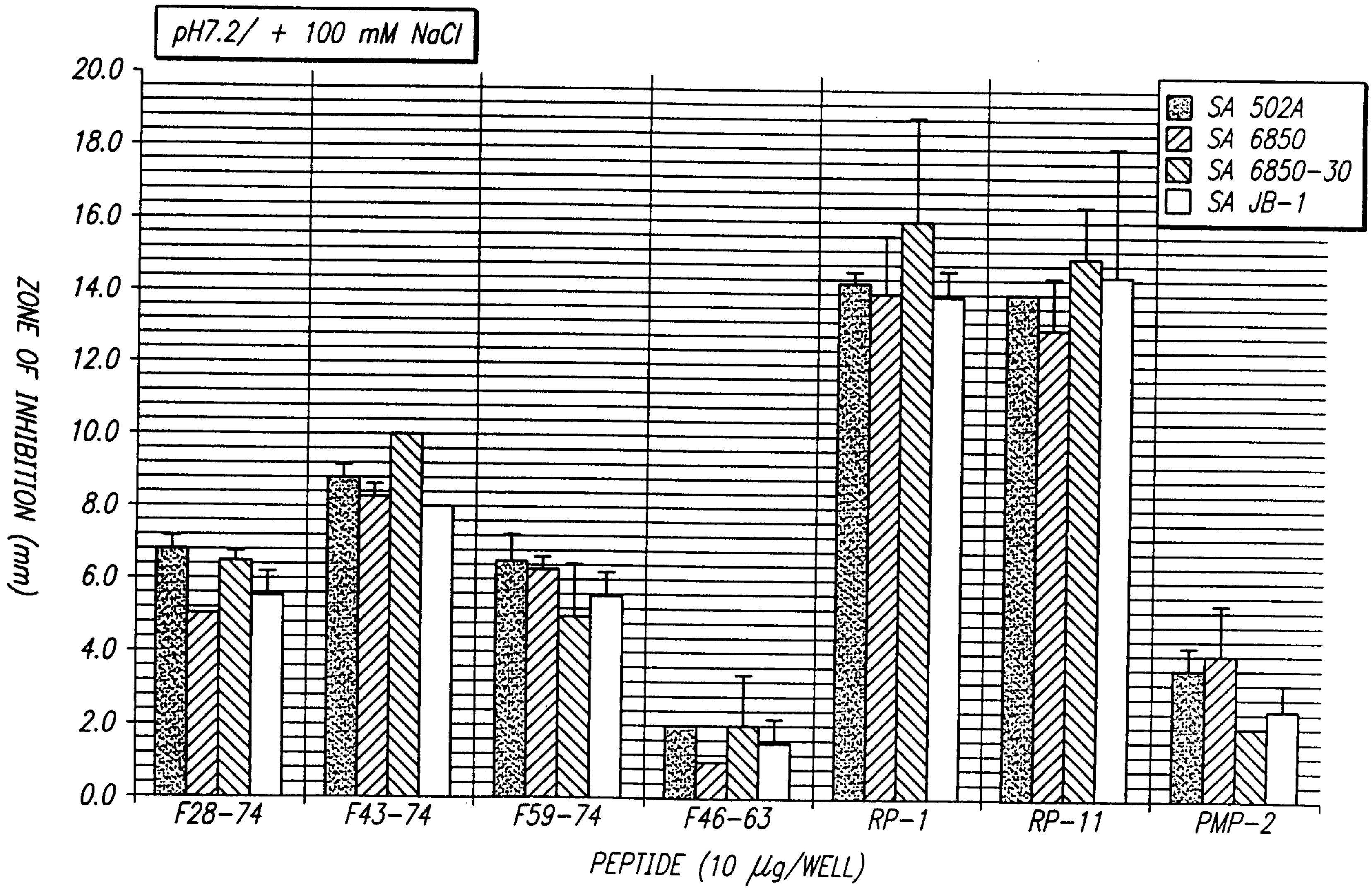
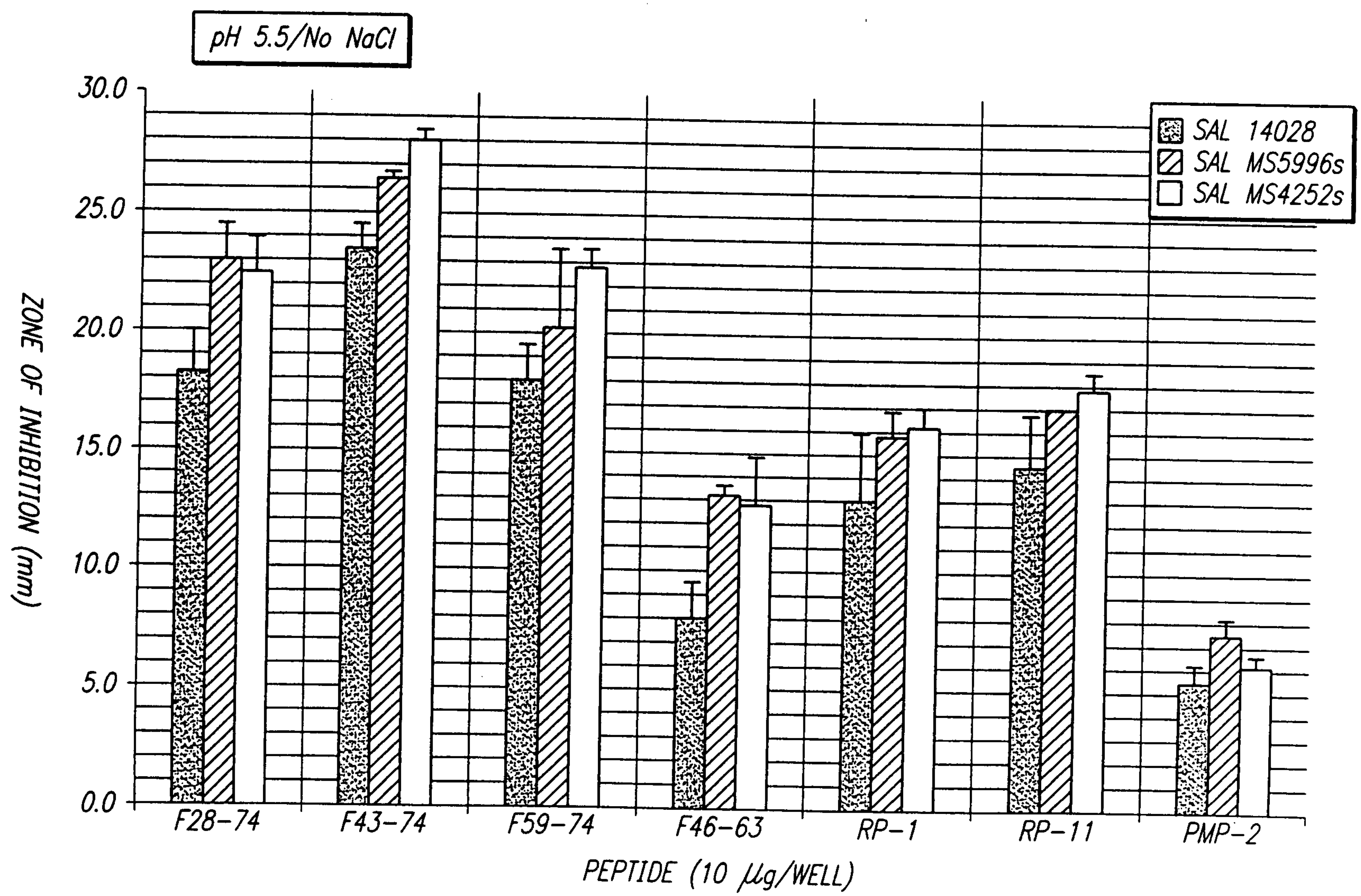
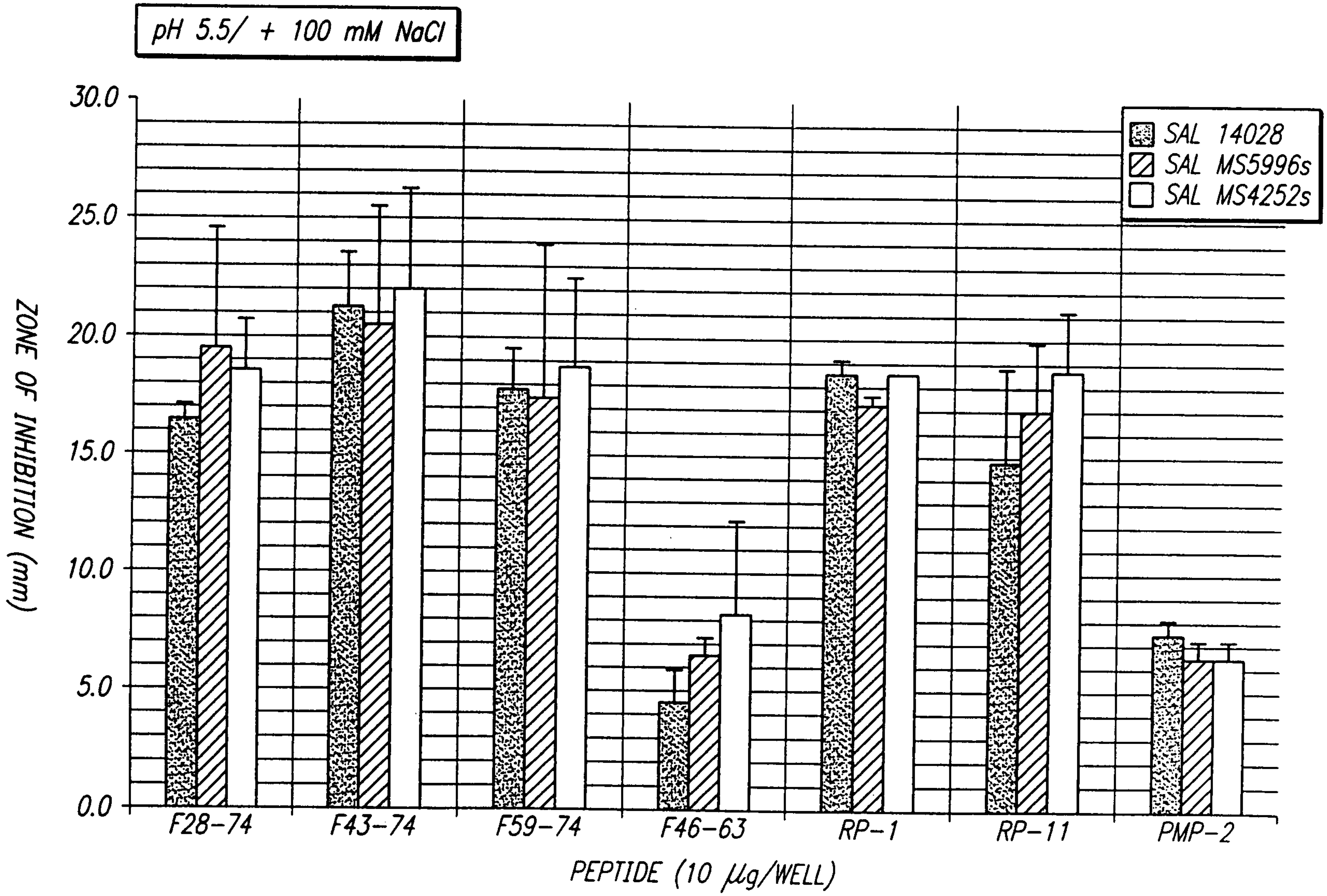

pH7.2/ + 100 mM NaCl
ZONE OF INHIBITION (mm)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
SA 502A
SA 6850
SA 6850-30
SA JB-1
F28-74
F43-74
F59-74
F46-63
RP-1
RP-11
PMP-2
PEPTIDE (10 μg/WELL)

FIG. 8



pH 5.5/No NaCl
SAL 14028
SAL MS5996s
SAL MS4252s
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ZONE OF INHIBITION (mm)
F28-74
F43-74
F59-74
F46-63
RP-1
RP-11
PMP-2
PEPTIDE (10 μg/WELL)

FIG. 9



pH 5.5/ + 100 mM NaCl
SAL 14028
SAL MS5996s
SAL MS4252s
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ZONE OF INHIBITION (mm)
F28-74
F43-74
F59-74
F46-63
RP-1
RP-11
PMP-2
PEPTIDE (10 μg/WELL)

00528 0.9 1 8 8 4 9 6 0

FIG. 10

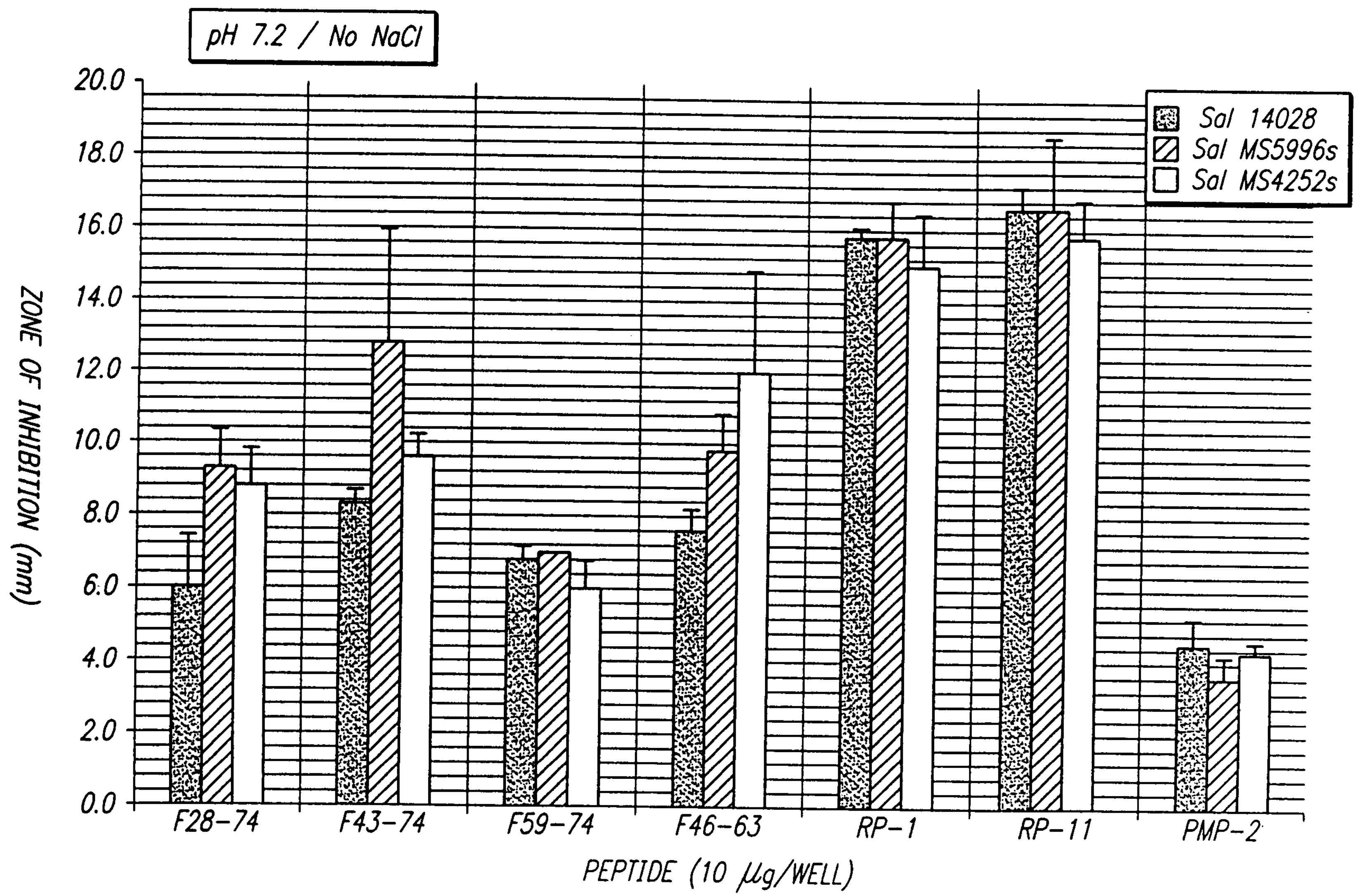


FIG. 11

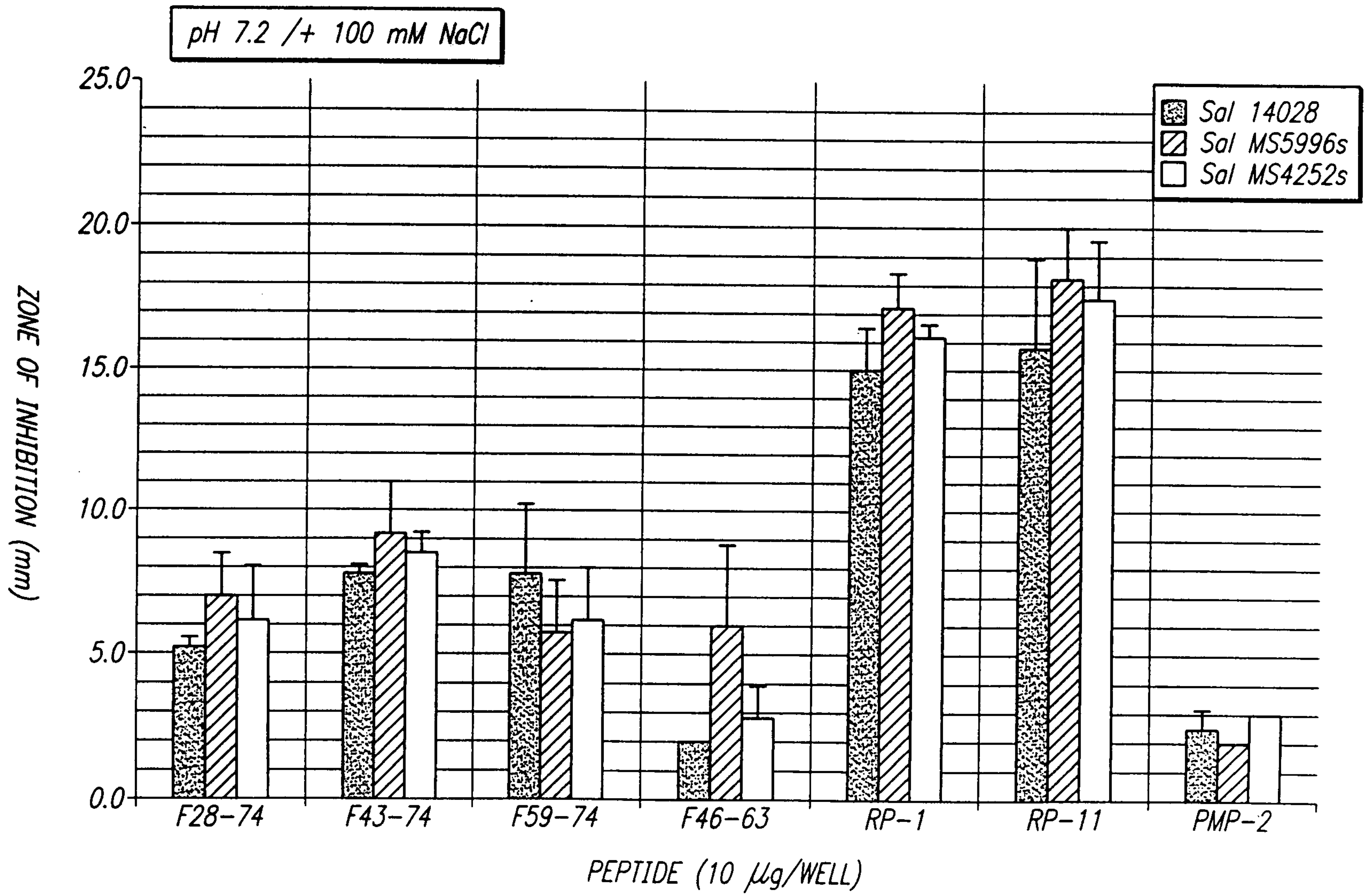

pH 7.2 /+ 100 mM NaCl
ZONE OF INHIBITION (mm)
25.0
20.0
15.0
10.0
5.0
0.0
Sal 14028
Sal MS5996s
Sal MS4252s
F28-74
F43-74
F59-74
F46-63
RP-1
RP-11
PMP-2
PEPTIDE (10 μg/WELL)

FIG. 12

PMP-2 RESIDUE:	10 20 30 40 50 60 70	MW (Da)
1-74	H_2N-SDDPKESEGDLHCVCVKTTSLVRPRHITNLELIKAGGHCPTANLIATKKNGRKLCLDLQAALYKKKIIKKLLES-COOH	7728
1-37	H_2N-SDDPKESEGDLHCVCVKTTSLVRPRHITNLELIKAGG-COOH	3805
38-74	H_2N-HCPTANLIATKKNGRKLCLDLQAALYKKKIIKKLLES-COOH	3923
1-15	H_2N-SDDPKESEGDLHCVC-COOH	1544
13-27	H_2N-CVCVKTTSLVRPRHI-COOH	1619
25-39	H_2N-RHITNLELIKAGGHC-COOH	1572
37-51	H_2N-GHCPTANLIATKKNG-COOH	1443
49-63	H_2N-KNGRKLCLDLQAALY-COOH	1614
60-74	H_2N-AALYKKKIIKKLLES-COOH	1653

FIG. 13

	PBS	N-f-MLF	rPF-4	rPMP-2	PMP-2(46-63)	RP-1
MEAN	1.1	2.6	2.2	2.3	1.4	1.5
S.D.	0.3	0.6	0.2	0.3	0.2	0.4

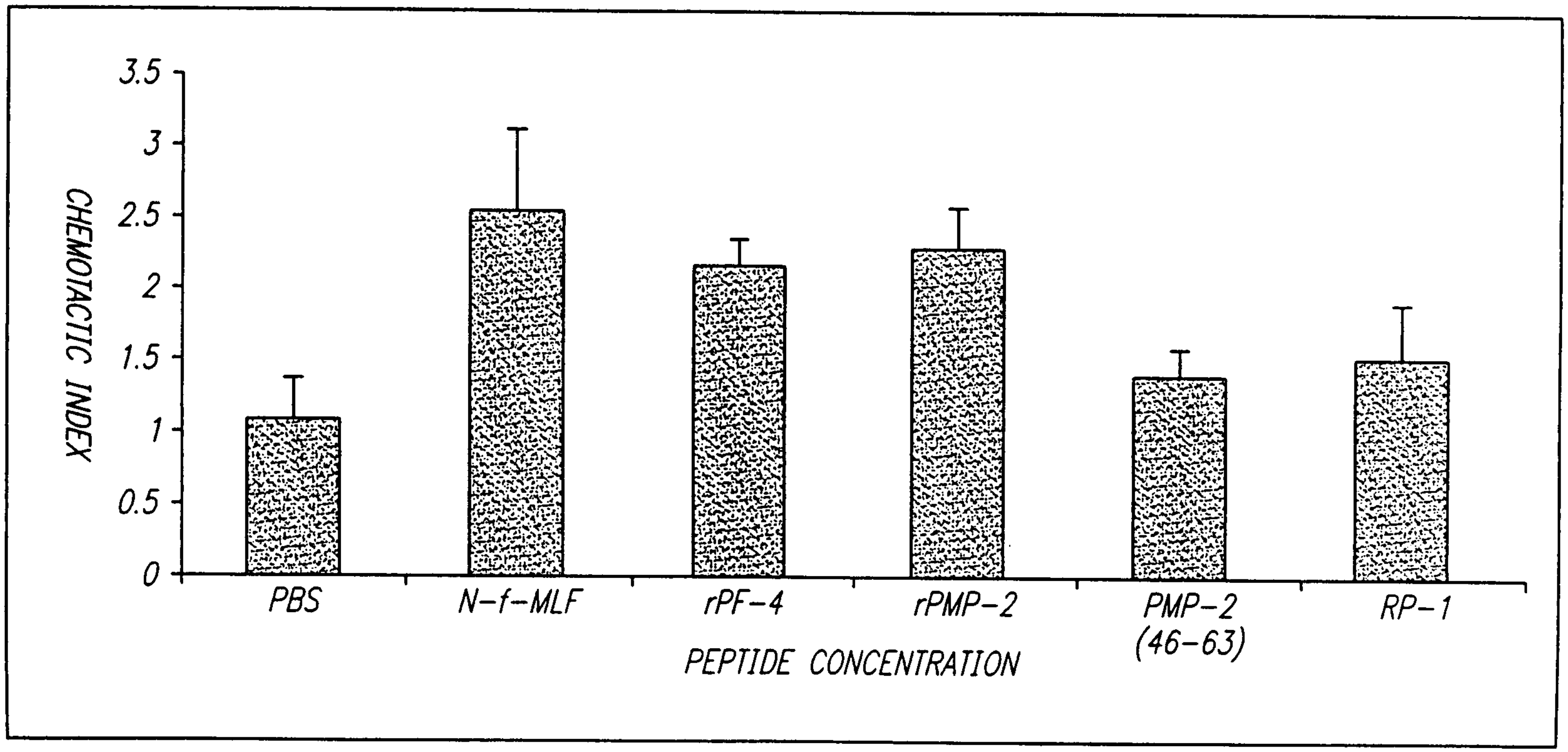
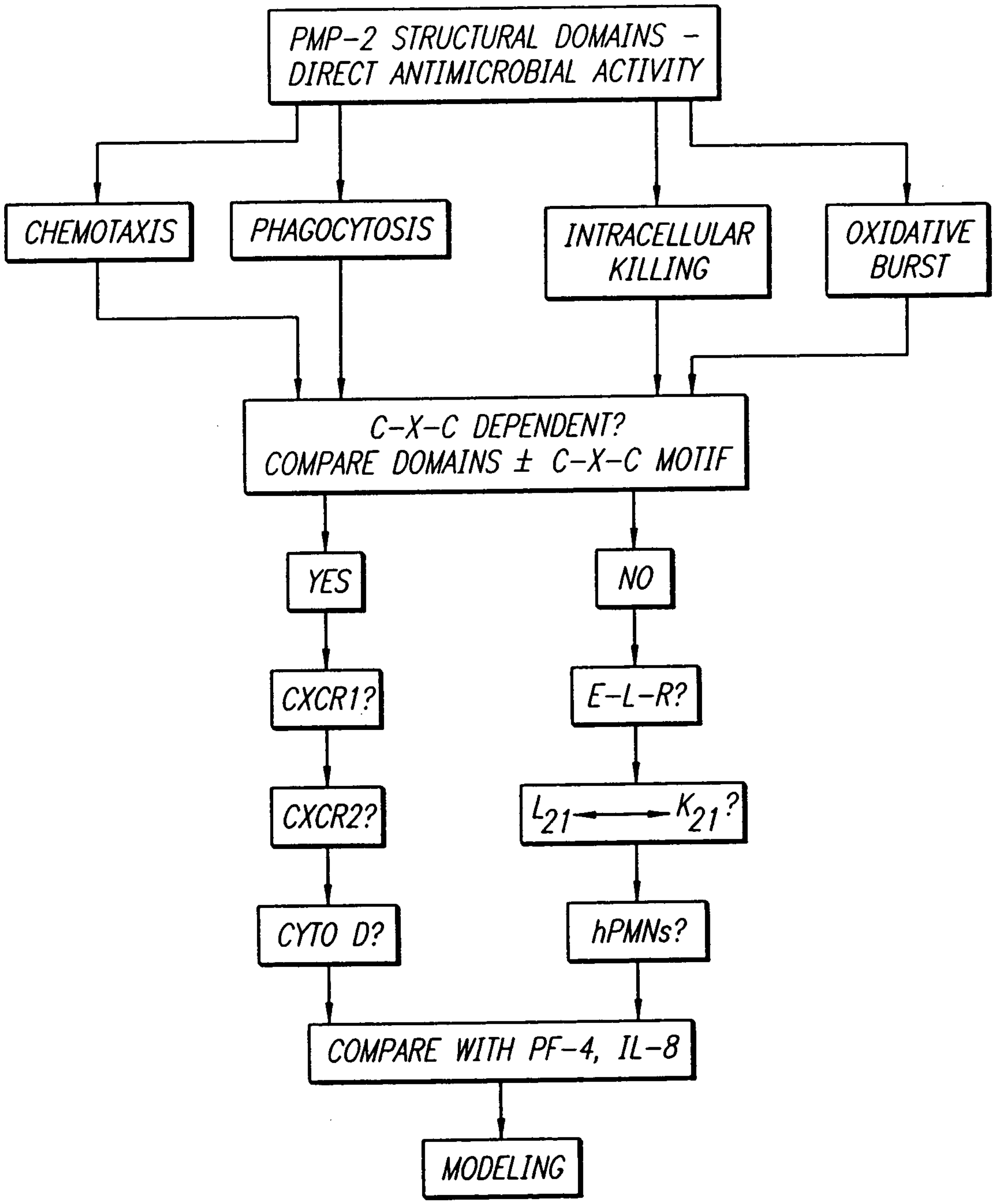


FIG. 14


PMP-2 STRUCTURAL DOMAINS - DIRECT ANTIMICROBIAL ACTIVITY
CHEMOTAXIS
PHAGOCYTOSIS
INTRACELLULAR KILLING
OXIDATIVE BURST
C-X-C DEPENDENT? COMPARE DOMAINS ± C-X-C MOTIF
YES
NO
CXCR1?
E-L-R?
CXCR2?
L_{21} ↔ K_{21}?
CYTO D?
hPMNs?
COMPARE WITH PF-4, IL-8
MODELING

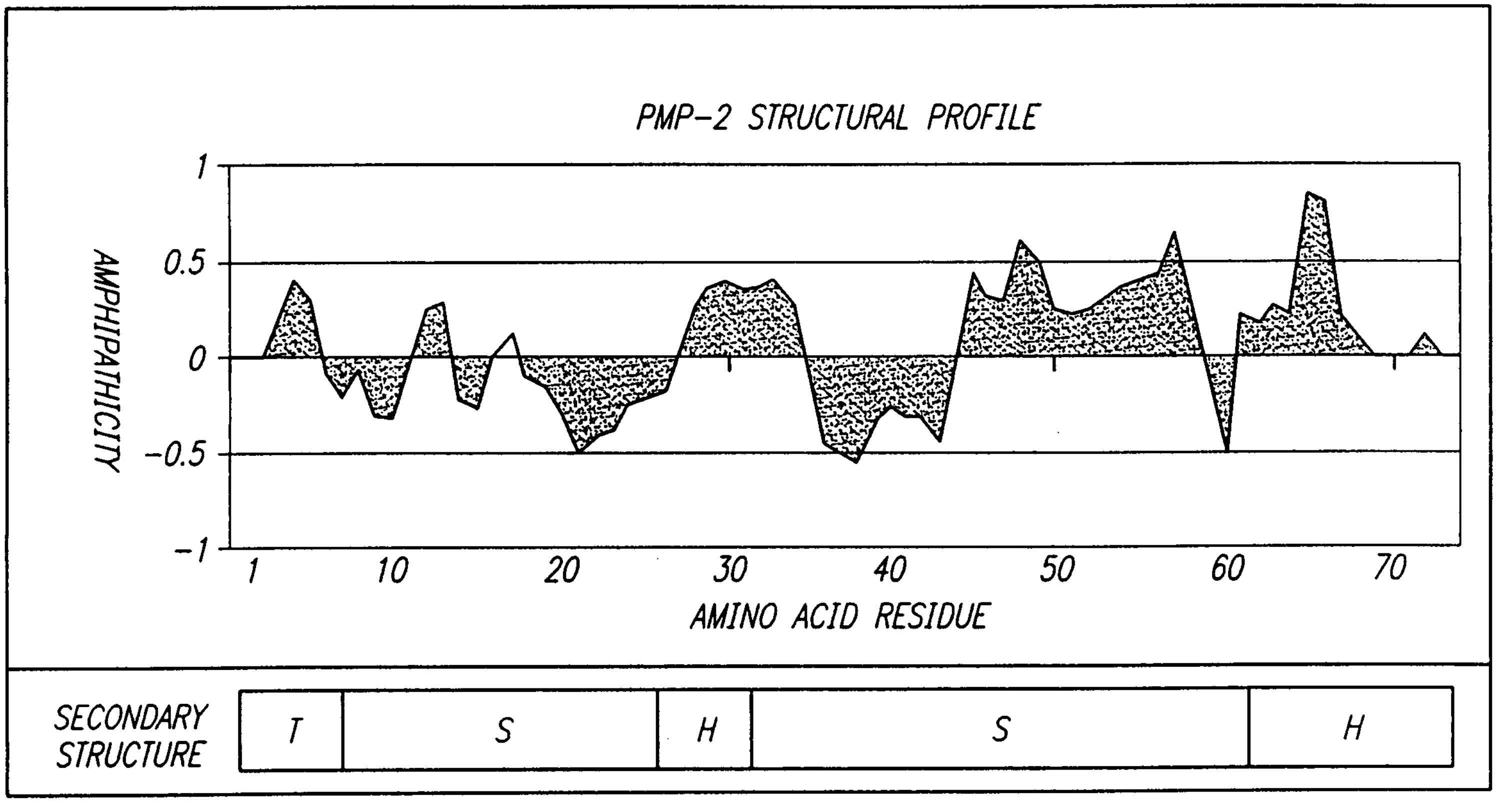
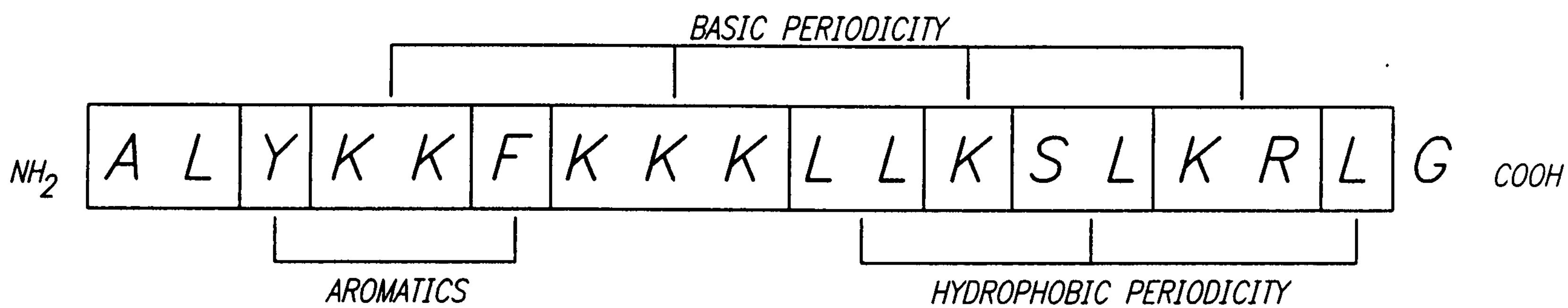
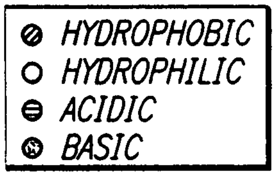

PMP-2 STRUCTURAL PROFILE
AMPHIPATHICITY
1
0.5
0
-0.5
-1
1
10
20
30
40
50
60
70
AMINO ACID RESIDUE
SECONDARY STRUCTURE
T
S
H
S
H

FIG. 15

FIG. 16


BASIC PERIODICITY
NH2
A L Y K K F K K K L L K S L K R L G
COOH
AROMATICS
HYDROPHOBIC PERIODICITY


HYDROPHOBIC
HYDROPHILIC
ACIDIC
BASIC

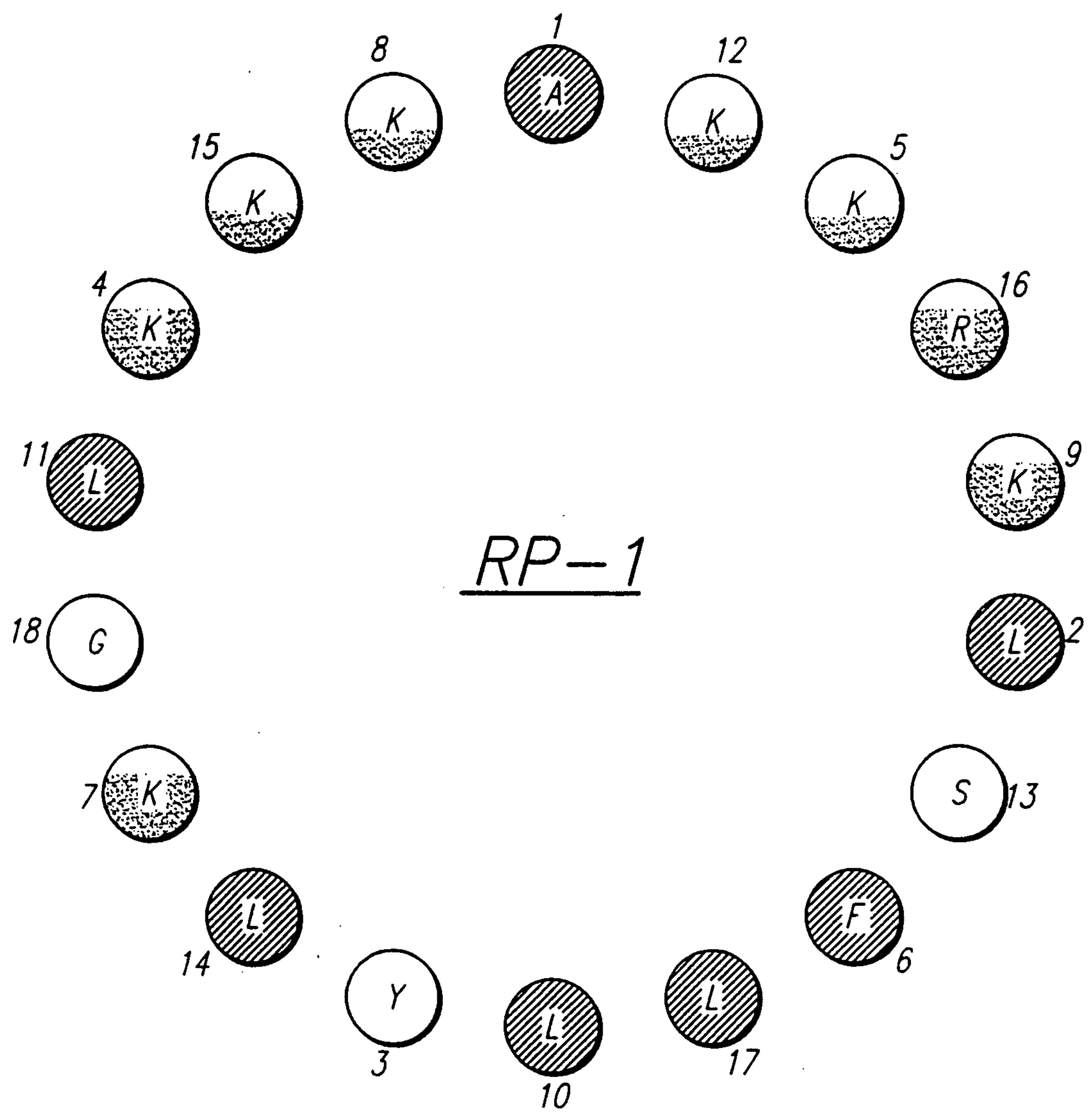
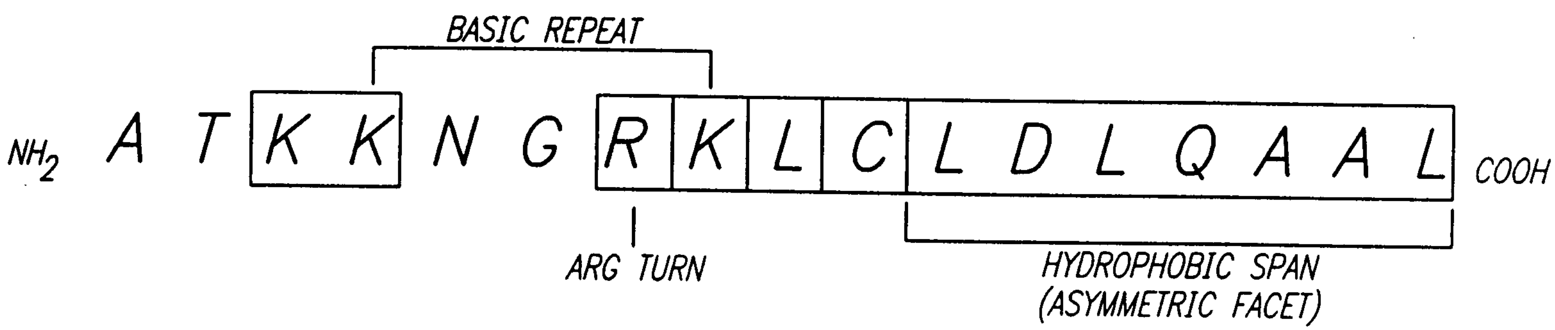

RP—1
1 A
12 K
5 K
16 R
9 K
2 L
13 S
6 F
17 L
10 L
3 Y
14 L
7 K
18 G
11 L
4 K
15 K
8 K

FIG. 17

FIG. 18


BASIC REPEAT
NH_2 A T K K N G R K L C L D L Q A A L COOH
ARG TURN
HYDROPHOBIC SPAN
(ASYMMETRIC FACET)

- HYDROPHOBIC
- HYDROPHILIC
- ACIDIC
- BASIC

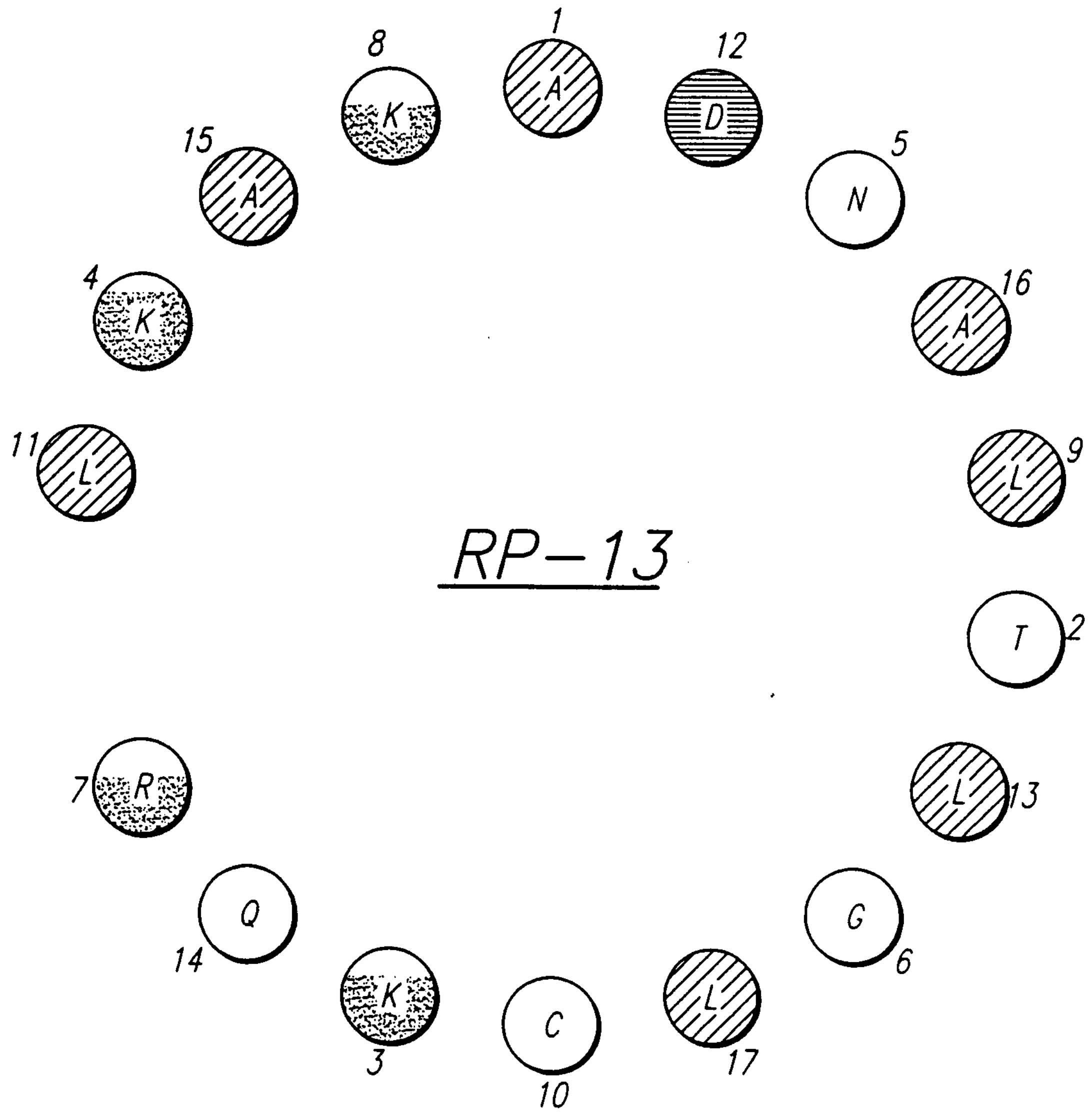


FIG. 19

GEOMETRIC MEAN MIC_{100}

	NUTRIENT BROTH				MUELLER-HINTON BROTH			
	pH 5.5		pH 7.2		pH 5.5		pH 7.2	
PATHOGEN	50%	100%	50%	100%	50%	100%	50%	100%
SA	70.7	100	3.1	3.1	100	>100	6.3	25
SE	6.3	8.8	1.6	3.1	1.6	3.1	25	25
EF	50	>100	25	50	>100	>100	25	35
SM	35.4	35.4	1.6	3.1	6.1	25	6.3	6.3
EC	25	25	12.5	12.5	12.5	25	12.5	12.5
PA	8.8	12.5	6.3	6.3	3.1	25	12.5	0.8
CA	17.7	35.4	6.3	12.5	>100	100	25	25
CN	12.5	25	1.6	1.6	>100	1.6	3.1	6.3

ORGANISM INOCULUM = 1×10^5 CFU/ml; LOGARITHMIC-PHASE CELLS
PEPTIDE CONCENTRATION = 10 μg/ml; (4.6 nmoles / ml ; 4.6 μM)
INCUBATION 37°C, AMBIENT CO_2; MIC_{100} READ AT 24HR ($n \geq 2$)

FIG. 20

GEOMETRIC MEAN MIC_{100}

	NUTRIENT BROTH				MUELLER-HINTON BROTH			
	pH 5.5		pH 7.2		pH 5.5		pH 7.2	
PATHOGEN	50%	100%	50%	100%	50%	100%	50%	100%
SA	1.6	8.8	6.3	8.8	25	100	>100	>100
SE	3.1	3.1	3.1	3.1	3.1	3.1	>100	>100
EF	6.3	6.3	12.5	25	25	100	100	>100
SM	3.1	1.6	3.1	3.1	12.5	50	12.5	>100
EC	12.5	25	6.3	12.5	100	>100	>100	25
PA	4.4	8.8	6.3	12.5	50	100	100	100
CA	6.3	8.8	6.3	6.3	25	100	25	50
CN	1.6	1.6	2.2	1.6	3.1	1.6	3.1	3.1

ORGANISM INOCULUM = 1×10^5 CFU/ml; LOGARITHMIC-PHASE CELLS
PEPTIDE CONCENTRATION = 10 μg/ml; (4.3 nmoles / ml ; 4.3 μM)
INCUBATION 37°C, AMBIENT CO_2; MIC_{100} READ AT 24HR ($n \geq 2$)

FIG. 21

GEOMETRIC MEAN MIC_{100}

	NUTRIENT BROTH				MUELLER–HINTON BROTH			
	pH 5.5		pH 7.2		pH 5.5		pH 7.2	
PATHOGEN	50%	100%	50%	100%	50%	100%	50%	100%
SA	1.6	6.3	3.1	2.2	50	100	>100	>100
SE	1.6	1.6	1.6	1.6	3.1	3.1	>100	>100
EF	2.2	3.1	17.7	35.4	50	100	>100	>100
SM	0.2	0.4	1.6	2.2	25	50	6.3	6.3
EC	6.3	8.8	3.1	6.3	100	>100	12.5	25
PA	1.6	3.1	3.1	6.3	12.5	100	100	>100
CA	4.4	4.4	3.1	4.4	25	50	12.5	12.5
CN	2.2	0.78	1.6	1.6	1.6	3.1	6.3	3.1

ORGANISM INOCULUM = 1×10^5 CFU/ml; LOGARITHMIC–PHASE CELLS
PEPTIDE CONCENTRATION = 10 μg/ml; (5.9 nmoles / ml ; 5.9 μM)
INCUBATION 37°C, AMBIENT CO_2; MIC_{100} READ AT 24HR ($n \geq 2$)

FIG. 22

GEOMETRIC MEAN MIC_{100}

	NUTRIENT BROTH				MUELLER-HINTON BROTH			
	pH 5.5		pH 7.2		pH 5.5		pH 7.2	
PATHOGEN	50%	100%	50%	100%	50%	100%	50%	100%
SA	19.8	25	>100	>100	100	>100	>100	>100
SE	5	6.3	>100	>100	100	6.25	100	>100
EF	12.5	25	>100	>100	100	100	100	>100
SM	6.3	19.8	>100	>100	50	100	100	>100
EC	12.5	25	>100	>100	50	>100	100	100
PA	9.9	19.8	>100	>100	50	100	100	>100
CA	25	39.7	12.5	50	>100	>100	>100	>100
CN	12.5	12.5	12.5	25	25	100	50	>100

ORGANISM INOCULUM = 1×10^5 CFU/ml; LOGARITHMIC-PHASE CELLS
PEPTIDE CONCENTRATION = 10 μg/ml; (5.4 nmoles / ml ; 5.4 μM)
INCUBATION 37°C, AMBIENT CO_2; MIC_{100} READ AT 24HR ($n \geq 2$)

FIG. 23

pH 7.2

PEPTIDE	ANTIMICROBIAL ACTIVITY †								TOXICITY ‡	
	SA	SE	EF	SM	EC	PA	CA	CN	RBC_{Hg}	HUVEC
RP-1	3.1	3.1	25	3.1	12.5	6.3	12.5	6.3	>98%	<5%
RP-2	6.3	6.3	25	1.6	100	25	>100	12.5	95%	5%
RP-3	3.1	3.1	50	1.6	6.3	25	12.5	1.6	95%	5%
RP-4	12.5	4.4	50	4.4	>100	50	12.5	3.1	97%	7%
RP-5	8.8	3.1	50	4.4	100	25	17.7	4.4	95%	6%
RP-7	70.7	12.5	50	25	100	>100	50	25	85%	12%
RP-8	6.3	3.1	25	3.1	12.5	12.5	6.3	1.6	90%	7%
RP-11	6.3	1.6	35.4	2.2	6.3	6.3	4.4	3.1	87%	8%
RP-13	>100	>100	>100	>100	>100	>100	>100	>100	94%	5%

ORGANISM INOCULUM = 1×10^5 CFU/ml; LOGARITHMIC-PHASE CELLS
PEPTIDE CONCENTRATION = 10 μg/ml; 37°C, AMBIENT CO_2; 24 OR 48HR
† GEOMETRIC MEANS OF MIC_{100} ($n \geq 2$); ‡ IN VITRO TOXICITY MARKERS

FIG. 24

pH 5.5

PEPTIDE	ANTIMICROBIAL ACTIVITY †								TOXICITY ‡	
	SA	SE	EF	SM	EC	PA	CA	CN	RBC_{Hg}	HUVEC
RP–1	70.7	6.3	50	35.4	25	8.8	17.7	12.5	ND	ND
RP–2	>100	12.5	100	12.5	>100	100	>100	50	ND	ND
RP–3	100	>100	50	12.5	100	100	25	25	ND	ND
RP–4	8.8	2.2	50	6.3	70.7	17.7	25	3.1	ND	ND
RP–5	4.4	0.4	25	0.8	50	8.8	12.5	3.1	ND	ND
RP–7	100	25	100	50	>100	100	100	12.5	ND	ND
RP–8	3.1	3.1	6.3	1.6	12.5	8.8	8.8	3.1	ND	ND
RP–11	3.1	1.6	25	0.4	12.5	3.1	6.3	3.1	ND	ND
RP–13	12.5	6.3	25	19.8	25	19.8	12.5	6.3	ND	ND

ORGANISM INOCULUM = 1×10^5 CFU/ml; LOGARITHMIC–PHASE CELLS

PEPTIDE CONCENTRATION = 10 μg/ml; 37°C, AMBIENT CO_2; 24 OR 48HR

† GEOMETRIC MEANS OF MIC_{100} ($n \geq 2$); ‡ IN VITRO TOXICITY MARKERS

FIG. 25